AGE-AT-DEATH ESTIMATION USING CEMENTOCHRONOLOGY IN THERMALLY ALTERED TEETH

by

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AGE-AT-DEATH ESTIMATION USING CEMENTOCHRONOLOGY IN THERMALLY ALTERED TEETH

Abstract

Macroscopic age-at-death estimations provide age ranges that give broad and often insufficient insight to an individual's chronological age. Accurate age estimations can become more complex for a forensic anthropologist if the unknown individual has been subjected to extreme heat from an assortment of scenarios that include but are not limited to structure fires, airplane crashes, automobile accidents, and attempts to conceal evidence of homicide. Due to their high mineral content and sequestered placement in the jaw, teeth have the ability to be one of the best-preserved human tissue remains in extreme heat situations (Beach, Passalacqua, & Chapman, 2015). Cementochronology utilizes the cementum, the mineralized covering of a tooth root, as an aid in estimating an individual's age (Colard, et al., 2015). The utilization of cementochronology is one of the most accurate ways to estimate age-at-death due to the countable cementum annulations found in the cross-sections directly correlating to the individuals age (Wittwer-Backofen, 2004). This study aims to provide an in-depth analysis of thermal alteration to human teeth by various accelerants when utilizing the cementochronology method to build a biological profile. The sample in this study consists of 36 teeth from both male and female donors from all odontological positions ranging in age from 9-87 years. Three accelerants with varied volumes were used to determine if the readability of cementum annulations can still remain accurate after alteration. Results indicated that the type of tooth had significant impact on the ability to count annulations. Annulations were able to be read and estimated after alteration with all three accelerants used in this study. However, acetone yielded the lowest results with the most severe alterations. A novel formula was developed to help approximate the amount of cementum annulations found within each sample. This formula was found to yield estimated tooth cementum annulations that were highly correlated with the actual ages of the individuals from whom the samples were from. After thermal alteration of teeth with accelerants it was found that cementochronology is an accurate and helpful tool for estimating age-at-death in unknown individuals.

Keywords: Cementochronology, Thermal Alteration, Age Estimation

Introduction

When a forensic anthropologist is building a biological profile, approximating an accurate age estimation is essential to narrowing down the list of potential missing person comparisons. Traditionally, age estimation is performed through a variety of skeletal assessments that helps correlate biological or skeletal age with chronological age. Chronological age, or the length of time an individual has been alive, is the age that forensic anthropologists work towards estimating most accurately when building a biological profile (Christensen, Passalacqua, & Bartelink, 2014). However, for adults, this is often done through skeletal estimation and the degeneration processes that are consistent in the bones of humans resulting in an accurate age interval that is exceedingly less precise.

Cementochronology is a technique that was first developed for nonhuman mammalian species. This technique involves counting incremental lines in tooth root cementum to estimate age and research has found that the methodology can be applied to age-at-death estimations in human remains (Colard et al., 2015). The number of tooth cementum annulations counted in an individual's dentition can be added to the known estimated age of tooth eruption to arrive at a more accurate age-at-death estimation. This approach becomes increasingly important if other areas of the body that can be used to estimate age are damaged, degraded, or missing especially when related to the field of forensic science. In situations such as these it is also increasingly common to find unknown individuals who have fallen victim to intentional or accidental extreme heat situations.

The primary goal of this research is to aid forensic anthropological age estimations by confirming previous research using cementochronology on human teeth. In addition, verifying a precise, accurate chronological age range can be determined. While numerous studies have been conducted utilizing cementum annulations in human dentition, there has not been research specifically focused on using accelerants to alter the teeth in order to mimic intentional arson situations, accidental fires where accelerants play a factor, or mass disaster situations where accelerants are present. The other primary focus of this research is to determine if this method can be applied to thermally altered teeth by accelerants or otherwise. This research aims to find if the cementum annulations found in teeth can still be observed and used to estimate age after being subjected to a variety of accelerants that are often found in intentional and accidental fire scenarios. Based on the previous research conducted and an understanding of the dental anatomy, the cementum annulations may have exhibit alteration, but not to the extent that will be immeasurable with the accelerant temperatures.

Background Information

Forensic Anthropological Age Estimations

Traditionally, age is estimated through the skeleton and is done so through the comprehensive understanding of the nature, sequence, and timing of skeletal changes over the course of an average lifetime, as well as understanding the relationship between these processes and chronological measures. These understandings work together to estimate an individual’s biological age and chronological age. Biological age and chronological age are not perfectly correlated, and this is due to a variety of factors of the aging process and how it differs between individuals. Biological age varies as a function of genetic, nutrition, environmental factors, and more as chronological age is measured by time (Christensen, Passalacqua, & Bartelink, 2014). For forensic purposes, age is broken into two broad categories: juvenile, also referred to as subadult, and adult. Juveniles are classified as those ages in which the growth and development process is in progress and include embryonic, fetal, infant, child, and adolescent periods beginning from the first 8 weeks of intra-uterine life to 17-years-old. Adults, 17 years of age or older, are those whose ages occur during the mature and degenerative skeletal changes. The growth and development of the skeleton is considered complete when all permanent teeth have erupted and all epiphyses have fused (Christensen, Passalacqua, & Bartelink, 2014). Dental methods are most often used when estimating the age of juveniles as the formation, mineralization, and eruption of the deciduous and permanent dentition occur at known development times that are under strong genetic control. Dental development is highly correlated with chronological age and is often a more accurate and preferable method of age estimation in juveniles as opposed to methods related to bone development (Christensen, Passalacqua, & Bartelink, 2014). Dental methods for age estimation can be expanded and applied to all ages through microscopic analyses with methods such as cementochronology.

Accelerants

An accelerant, as defined by Almirall et al., is a substance that is often a petroleum-based product to speed up combustion of materials that do not readily burn (Almirall et al., 2004). These substances are used daily within motor vehicles, aircrafts, or in cooking appliances. However, if mishandled or used incorrectly, a fire resulting from the combustion of an accelerant can be devastating and deadly.

Acetone, while not a petroleum-based product, is an accelerant often found in arson situations. Acetone is a colorless, volatile, flammable organic solvent that occurs naturally in the environment while also commercially available. This product is most often used as a solvent in lacquers, varnishes, cosmetics, and in mixtures with other solvents. While it is a commercial product, it is highly flammable with an ignition temperature of 465.4 degrees Celsius. This temperature is low enough to be reached by all commercial ignition sources such as household lighters, matches, and (National Center for Biotechnology Information, 2021).

Lighter fuel, or butane, is a colorless liquid or vapor that is easily ignited that under prolonged exposure to fire or intense heat can violently explode. It is commercially used as fuel, an aerosol propellant, in lighters, and to make other chemicals. Due to the low ignition temperature of 25-38 degrees Celsius lighter fuel is ideal for commercial use (National Center for Biotechnology Information, 2021).

87 grade gasoline is an unleaded gasoline that is also referred to as octane. This type of gasoline is a colorless liquid that can be found at almost any gasoline station around the world and is used in most motor vehicles. Gasoline is a highly flammable liquid that is the most commonly identified ignitable liquid accelerant reported by American forensic laboratories for arson situations (Almirall et al., 2004). Gasoline can ignite at 280 degrees Celsius and is very unstable once burned (National Center for Biotechnology Information, 2021).

Tooth Cementum

Tooth cementum, referred to simply as cementum, is formed by mineralization and is laid on the roots of teeth in layers. Cementum undergoes appositional growth in which an alteration in the orientation of the layers can be seen in a light band and a dark band being laid down in a circa-annual rhythm when examined through a microscope. The regularity of this rhythm is well documented and is deemed reliable as the tooth cementum does not undergo alterations during its lifetime. This stability also remains over long postmortem changes and intervals making it ideal for forensic related casework (Wittwer-Backofen, 2004).

Cementum is a mineralized tissue that covers the tooth root dentin. The two major types of cementum that exist in human teeth are acellular or primary cementum and cellular or secondary cementum. Acellular cementum is located on the cervical portions of the roots and anchors the tooth to the alveolar bone whereas cellular cementum is located apical roots and plays a role in post-eruptive adjustment of tooth positioning (Zhao et al., 2016). During the formation of cellular cementum, cementoblasts secrete layers of cementoid into the extracellular matrix. As cementoid deposition progresses, cementoblasts can become embedded in the extracellular matrix and become cementocytes. While this process is similar to that of bone, cementum is avascular, or lacks blood vessels, and therefore does not undergo physiological remodeling, but rather grows continuously throughout life (Zhao et al., 2016).

Cementum consists of about 45-50% inorganic material and 50-55% organic material making cementum slightly softer than dentin (Chen and Liu, 2014). The cementum works as an attachment for the periodontal ligament, this in turn anchors the root of the tooth to the alveolar bone. This allows the tooth to maintain effective stabilization in the bones of the mandible and maxilla (Gocha & Schutkowski, 2013). As well, the structure of cementum is similar to that of compact bone. Both cementum and compact bone are composed of cells and mineralized extracellular matrix. However, cementum, unlike bone, does not have Haversian canals, blood vessels, or nerves. Throughout the life cycle of the tooth, a new layer of cementum is deposited to keep the attachment to the alveolar bone in place. However, unlike bone tissue that can be constantly rebuilt, cementum is only capable of repairing itself to a degree. This is because cementum has a stronger anti-absorption capacity compared to bones like those attached to the alveolar bone (Chen and Liu, 2014). Due to this, the analysis of tooth cementum becomes an ideal location for research as the lack of natural alteration to the cementum is infrequent compared to that in bone.

Cementochronology

Tooth cementum annulation analysis has been advocated as one of the most accurate methods for estimating age-at-death for human remains and has shown to have significantly lower error rates than that of morphological age estimation methods (Wittwer-Backofen, 2004). This type of analysis is done by cutting thin sections of the middle third area of the tooth root. These sections can be done by cutting an angle perpendicular to the exterior of the tooth root, but this method of sectioning is not applicable to molars. To keep methodology standardized if using teeth from all odontological positions, the samples should be sectioned perpendicular to the long axis of the tooth (Gocha & Schutkowski, 2013). The number of annulations found in the samples can then be counted manually and the mean of annulations counted can then be added to the age of tooth eruption to arrive at an estimated age-at-death. When performed correctly, tooth cementum annulation analysis' error range of individual age diagnosis can reach 0.8 years for young adults or 2-3 years for the whole age range (Wittwer-Backofen, 2004).

<u>Previous Research</u>

There are three main research studies pertaining to the conception and development of this project. These research studies include the use of cementochronology to estimate age in humans, the use of cementochronology in forensic contexts, and if cementochronology can be used to estimate age after remains have been subjected to extreme heat.

Wittwer-Backofen, et al. published a validation study to show that tooth cementum annulations can be used more reliably than other morphological or histological traits of the adult human skeleton in order to estimate age (Wittwer-Backofen, 2004). Their study was conducted using a large known-age sample of 363 teeth, where they found there was a high concordance between tooth cementum annulation age estimations and chronological age. Along with this, they found that sex differences, intraindividual correlations, and the effects of periodontal disease had no quantitative effect on the ability to count cementum annulations. They were able to assess the method's accuracy and show that the technique is successful in estimating age within 2.5 years to the known chronological age. It was concluded that using tooth cementum annulations is a reliable way to estimate age with smaller confidence intervals, providing individual ages with high probability in narrow age ranges.

Colard et al. published their study to work towards implementing cementochronology within forensic contexts as well as to establish a standard for how samples should be prepared in order accurately conduct tooth cementum annulation counting. In their study, they applied their certified protocol for cementochronology to nine anthropological cases from the Anthropology Department of Lille 2 University. They compared five anthropological age estimation methods and two dental age estimation methods to the data they obtained through tooth cementum age estimations. They concluded that once standardized, the cementochronology method is straightforward and does not rely as heavily on the experience required for age estimations using morphological methods. The authors argue that despite publications from Meinl et al., the cost of preparing one tooth is fast, affordable, and that the entire process can be conducted within half a day if the proper equipment is obtained (Meinl, 2008). As well, their results found that age estimations through cementochronology were both accurate and precise and are able to narrow down the age at death of the individual to be identified significantly. This method should become and continue to be an important tool and routine with forensic institutes when estimating age.

Gocha and Schutkowski (2013) published their study on how thermal alteration impacts the ability to estimate age when using tooth cementum annulations. In the study, teeth of known demographics were exposed to temperatures of 600, 800, and 1000°C. It was found that the cementum annulations survive thermal alteration, but their visibility is dependent on the exposure temperature. Teeth exposed to temperatures above 600°C do not yield accurate enough results to be of use in forensic investigations. However, due to the placement of teeth inside the jaw and the surrounding tissue, the results could differ making the application of tooth cementum annulations in thermally altered remains still of forensic value.

The importance of the research presented here is to add to the overall understanding of using cementochronology and to see if it can still be utilized in forensic contexts when teeth have been thermally altered via common accelerants. The design of this experiment was done in a manner to allow for direct comparisons between the volume of accelerant used during the thermal alteration process as well as the difference between the types of accelerants used.

Materials

1. Human Teeth (36 samples)
2. Klean Strip® Acetone
3. Zippo Lighter Fuel®
4. 87 Grade Gasoline
5. Distilled Water
6. Soft Bristled Toothbrush
7. Measuring Caliper
8. WESTWARD LCD, Infrared Thermometer, Single Dot Laser Sighting
9. IsoMet™ Saw with Diamond Wafering Blades
10. Buehler Cool 3, Cutting Fluid
11. Richard-Allan Scientific™ Mounting Medium
12. Petrographic Microscope Slides
13. PELCO® Geoslides Storage Box
14. 10 mL Crucibles and Crucible Lids
15. 10x10 Metal Mesh Sieve

16. 3mL Pipets
17. Aluminum Foil
18. Multi-Purpose Lighter
19. Castin' Craft® Clear Polyester Casting Resin
20. Castin' Craft® Catalyst for Casting Resin
21. BUYGO Silicone Epoxy Resin Molds
22. Popsicle Sticks
23. Metal Forceps
24. 50mL Beaker
25. Tongs
26. Gloves
27. Goggles
28. Timer
29. 12x12 Tile
30. Pro Grade Precision 220 Grit Fine Advanced Sanding Sheets
31. Imperial Wetordry 3000 Grit, Super Ultra Fine Grade Sandpaper
32. Petrographic Slide Mount
33. Digital Micrometer
34. Leica© DM750P Microscope
35. Leica© ICC50 HD Camera
36. Leica© LAS EZ Application Software
37. SAS® Studio
38. Microsoft® Excel®

Methods

Dental samples were collected from various oral surgery offices throughout the Las Vegas and Henderson, Nevada area. In total, 36 permanent teeth from 19 individuals were collected. All teeth were collected anonymously from both male and female donors, ranging in age from 9-87, see Figure 1. The average mean of ages from the teeth collected was 49.5 years with the standard deviation being 26.4 years, see Table 1 and 2. Teeth from all odontological positions were utilized, and each position of the tooth/teeth, sex of the patient, and age at extraction was documented via the oral surgeon or an assistant. Extra precaution was taken to exclude third molars from the sample as the age of eruption of third molars is highly variable (Kaitsas and Olivi, 2016). All teeth were blindly assigned identification numbers to ensure ignorance of sample origin during processing.

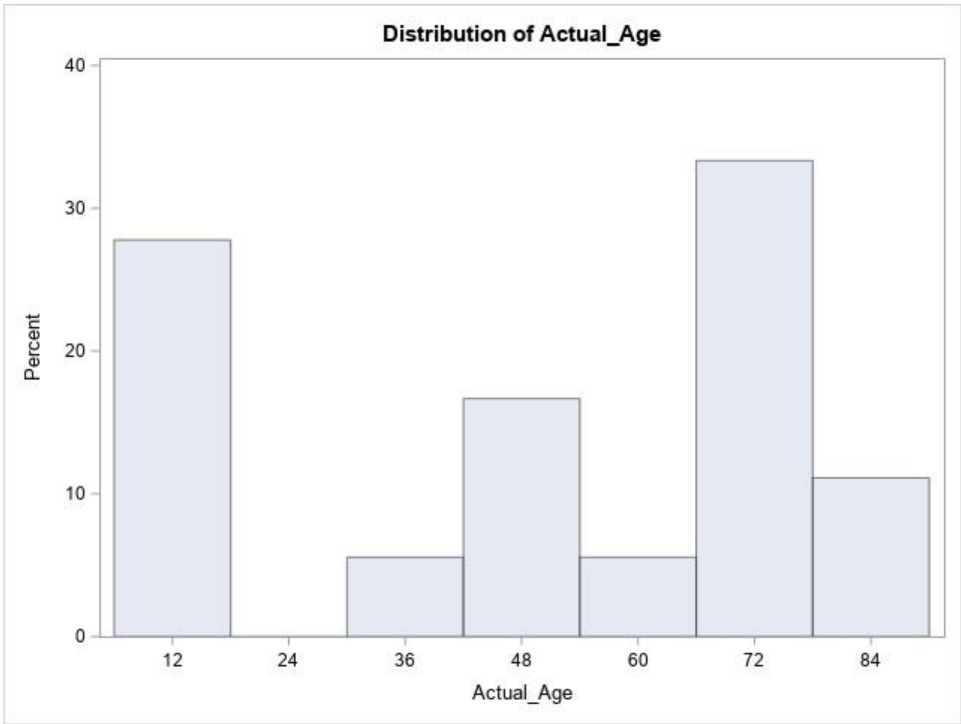

Figure 1: *Distribution of actual age of samples*

Table 1: *Ranges of samples*

Moments			
N	72	Sum Weights	72
Mean	49.5555556	Sum Observations	3568
Std Deviation	26.4398807	Variance	699.067293
Skewness	-0.3216026	Kurtosis	-1.3969706
Uncorrected SS	226448	Corrected SS	49633.7778
Coeff Variation	53.3540194	Std Error Mean	3.11596983

Table 2: *Standard statistical measurements of samples*

Basic Statistical Measures			
Location		Variability	
Mean	49.55556	Std Deviation	26.43988
Median	58.00000	Variance	699.06729
Mode	70.00000	Range	78.00000
		Interquartile Range	54.00000

Processing began with the initial cleansing of each tooth with distilled water and an extra soft bristled toothbrush to ensure the removal of any remaining adhering tissue or blood from extraction. Once thoroughly cleansed, the length and width of each tooth was measured with measuring calipers to the nearest millimeter to account for any shrinkage during experimentation. The 36 samples were then separated into groups of three to represent one accelerant to be further separated into groups of four. Four groups of teeth were created, and each group included at least one molar. In total, each accelerant had four groups of three teeth, totaling twelve teeth per accelerant and three teeth per assigned volume.

Thermal Alteration

A setup was created to hold each tooth in the center of the middle and outermost zone of the flame in order to achieve maximum exposure (see Figure 2 and 3). This setup included a 10mL crucible lid surrounded by tinfoil to hold the lid upright. A 10x10 metal mesh sieve was then placed over the opening of the crucible lid and the tooth was placed in the center of the sieve.

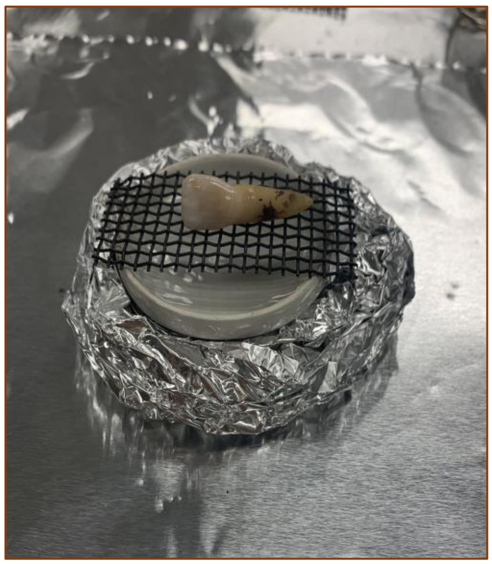

Figure 2: *Setup of materials for thermal alteration*

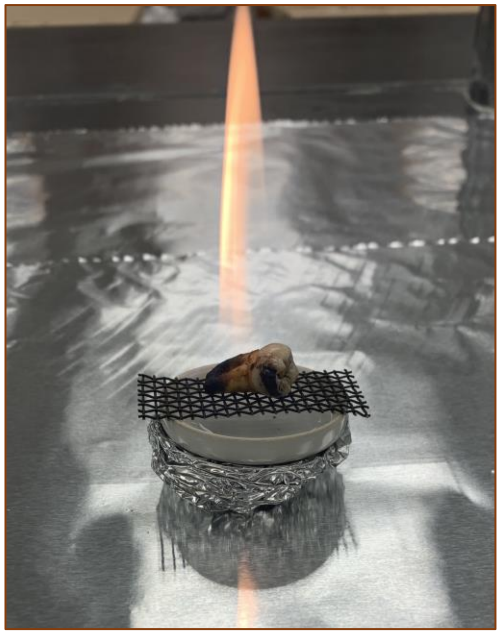

Figure 3: *Setup of materials during thermal alteration*

Experimentation began with igniting 1mL of Klean Strip® 100% Acetone with a multipurpose lighter and recording the time from ignition to extinguish as well as the maximum tooth temperature reached within the time frame. Temperature was recorded in degrees Celsius with a WESTWARD LCD Infrared Thermometer and time was recorded from ignition to extinguish on a stopwatch to the nearest second (see Figure 4). This was repeated with three teeth in total and continued with three teeth per milliliter until 4mL volume was reached. The process was then repeated with Zippo Lighter Fuel® and 87 Grade Gasoline ensuring to record the maximum tooth temperature reached (see Figure 5). Figure 4 demonstrates the distribution of time of tooth alteration for all accelerants at all volumes in seconds. Figure 5 is the distribution of maximum tooth temperature reached during alteration.

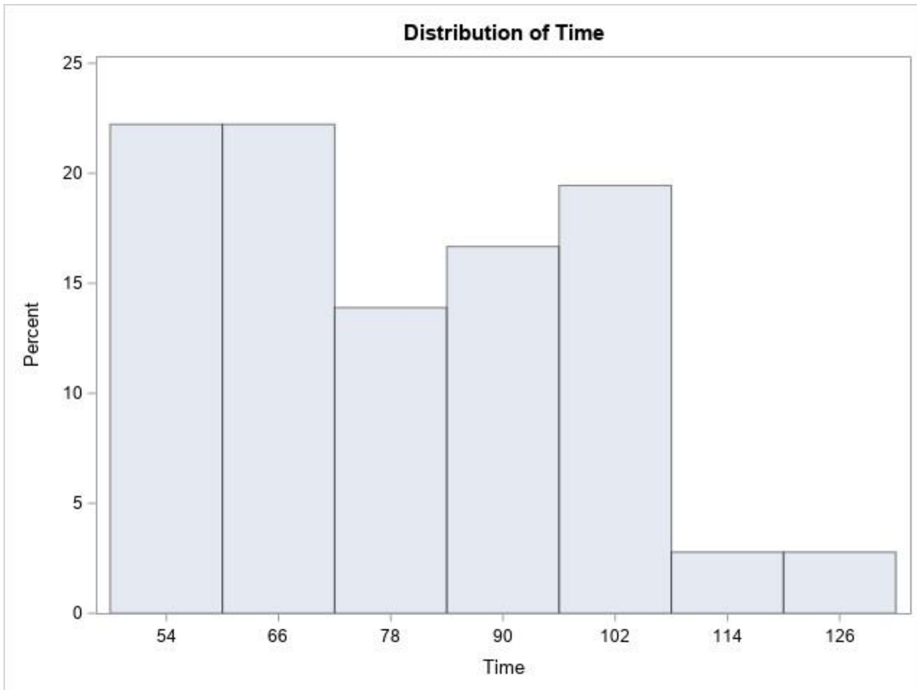

Figure 4: *Distribution of burning time recorded in seconds*

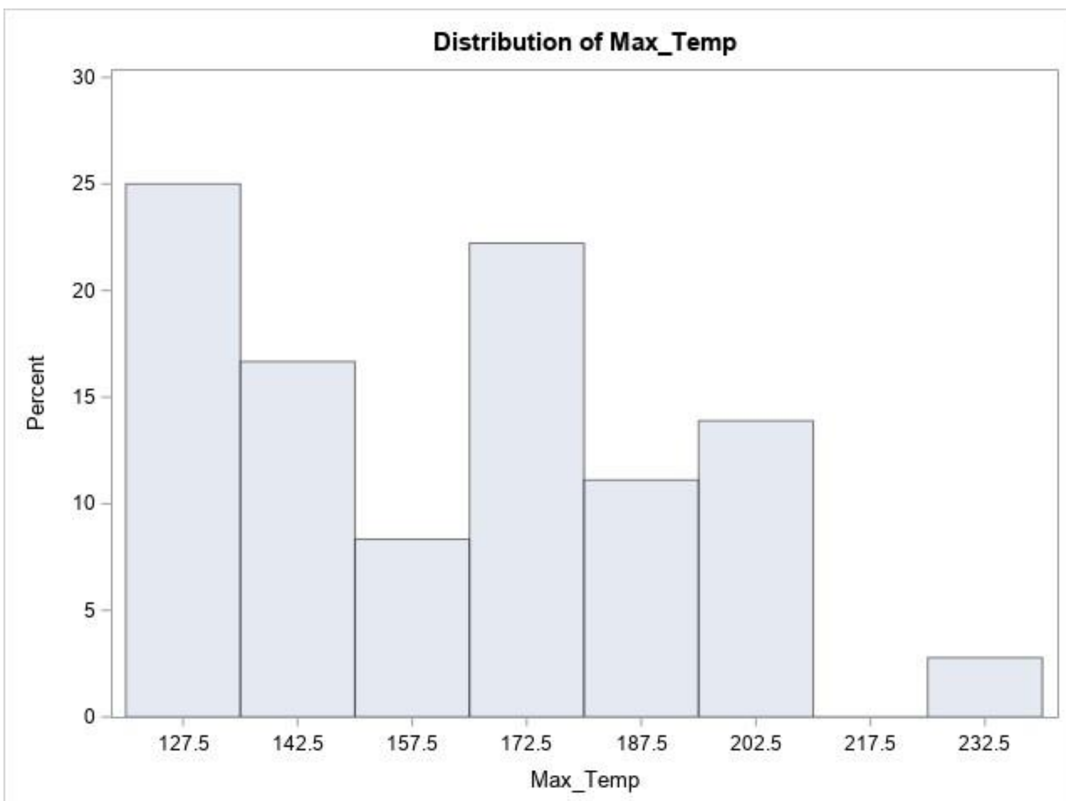

Figure 5: *Distribution of maximum tooth temperature*

After each tooth cooled, the dental ruminants were placed in silicone resin molds and embedded in Craft® Clear Polyester Casting Resin to maintain structural integrity during the thin sectioning. Each cast was left undisturbed for at least 24 hours to ensure complete curing of the resin. After the curing period, each cast was removed from the mold and each section was labeled with the corresponding number assigned at the beginning of the experimentation.

Cross Sectioning

Processing continued with the cross sectioning of each sample with an IsoMet™ Saw equipped with a diamond wafering blade (Buehler, Lake Bluff, IL). Sections were taken from the middle third of the tooth root (see Figure 6) perpendicular to the long axis of the tooth as to maintain standardization between multirooted and single-rooted teeth (Gocha & Schutkowski, 2013). The first cross-section was taken to separate the root from the crown of each tooth. The roots were then sectioned in half at the middle third, and the opposite side from the cut site was mounted to a Petrographic microscope slide via Richard-Allan Scientific™ Mounting Medium.

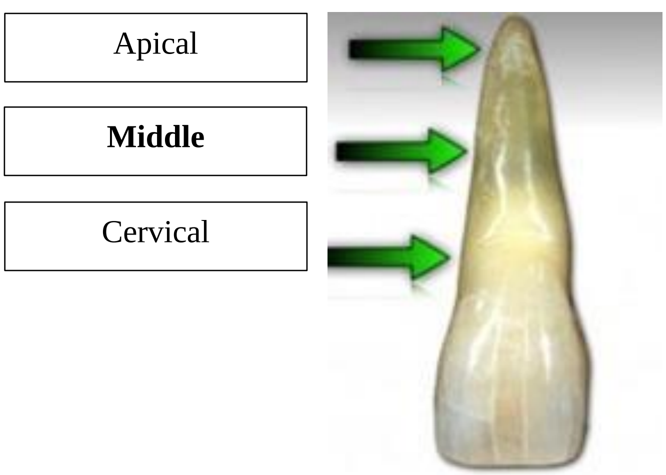

Figure 6: *Depiction of the middle of the tooth root where sectioning took place*

Manual Thinning

Manually thinning was completed in accordance with the process outlined by Maat, Bos, and Aarents (2001) with modifications made to fit the thinning of tooth samples. Thinning was completed with the use of 220 grit sandpaper for thinning sections to approximately 30-70 microns thick and were polished with 3000 grit sandpaper to remove any remaining grinding artifact. Once polishing was completed, each sample was measured again with a digital micrometer to ensure samples were below 70 microns in thickness.

Examination and Calculations

Each sample was then examined under a Leica© DM750P Petrographic Microscope with an attached Leica© ICC50 HD Camera. The microscope was then connected to a desktop computer so the Leica© LAS EZ Application Software could be utilized for examination, measurements, and counting of the annulations. Photographs of each sample were taken and processed within the Lecia© software. The entire sample was examined at 10x magnification in order to find clear areas of visibility. If an area of visibility was not able to be found due to the tooth having no remaining areas of cementum or the tooth being too burned, a photo was taken at 10x magnification (see Figure 7 and 8).

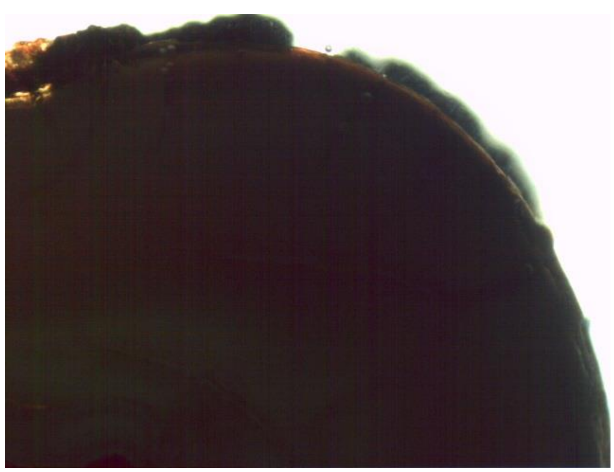

Figure 7: *Sample 23B, 4mL Acetone 10x magnification*

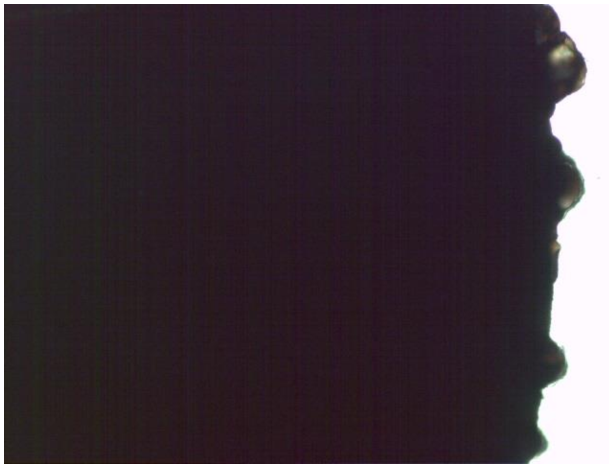

Figure 8: *Sample 26A, 4mL Lighter Fuel 10x magnification*

Once a visible area was found, magnification was increased to 40x magnification and a photograph was taken (see Figure 9 and 10). If the area was suitable for cementum annulation counting, deionized water was placed on the sample and a glass cover slip was adhered to the slide. The cementum was examined regardless of if the cementum was cellular, acellular, or contained cementocytes (see Figure 11). The magnification was increased to 100x magnification and adjustments were made. It was found that with the majority of the samples at 100x magnification, the illuminator would need to be at the highest brightness and the condenser would need to be lowered to increase contrast within the sample. Photographs were taken prior to measurements of the sample and were then further processed using the Leica© processing feature.

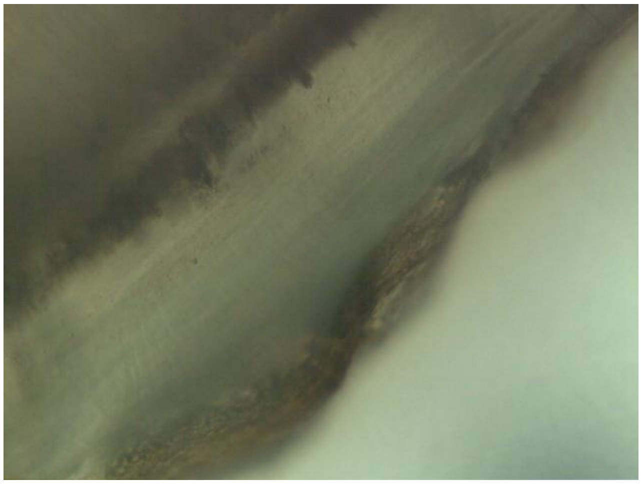

Figure 9: *Sample 2A, 2mL 87 Grade Gasoline 100x magnification no measurements*

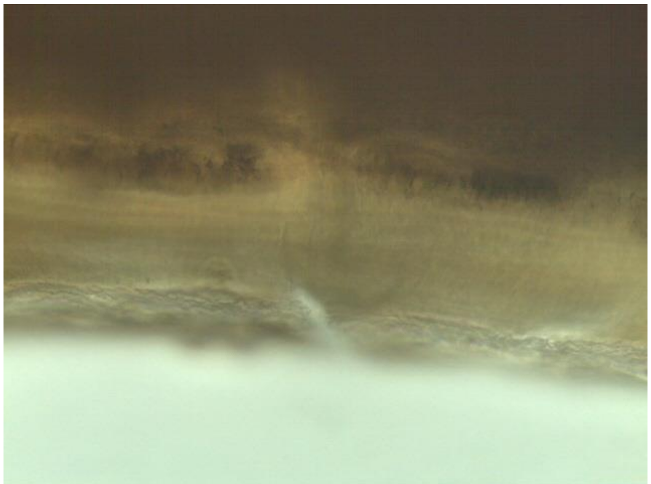

Figure 10: *Sample 20B, 4mL Acetone 100x magnification no measurements*

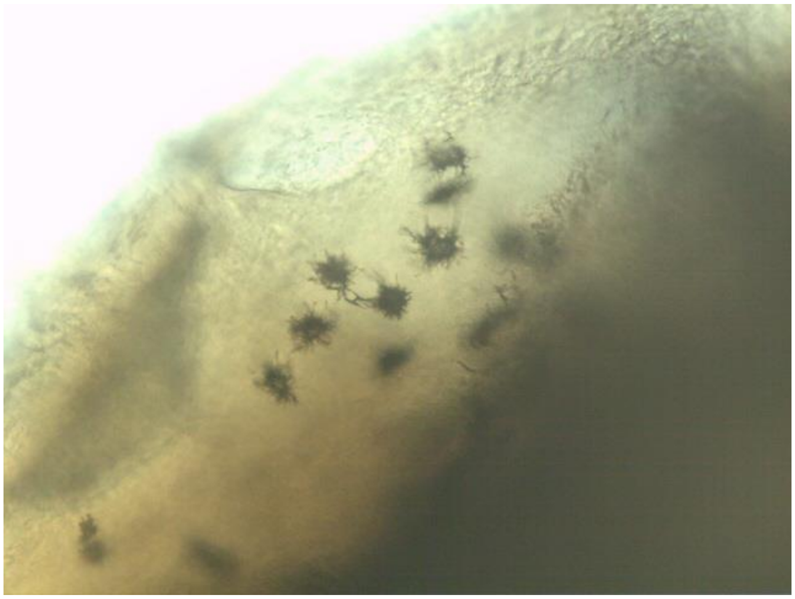

Figure 11: *Sample 5A 100x magnification of cementocytes*

The width of the cementum from the dentin-cemental junction to the surface of the cementum was measured in pixels in an area where the cementum appeared to run approximately parallel using the distance tool within the Leica© software (Gupta et al., 2014). Measurement of the width of three sets of clear, dark and light annulations were then measured (see Figure 12, 13, and 14). Using Formula 1, the total number of incremental lines in the total area of cementum was calculated where, X is the total width of the cementum and Y is the width of the cementum between three sets of incremental lines.

1) Number of incremental lines (n) = X/Y   2) n * Y = E (estimated age-at-death)

Using Formula 2, age estimation was calculated where n is the number of incremental lines in the entire area of cementum this number can then be multiplied by Y which is the width of three annulation lines to get to the estimated age-at-death (E). This two-step equation was created with the goal of creating a method of counting annulations when clear individual annulations are not present through the entire sample or individual annulations are present but not through the entire cementum area. This method can then in turn substitute the arduous individual counting of each annulation present, as well as aid in estimations when each individual annulation is not present.

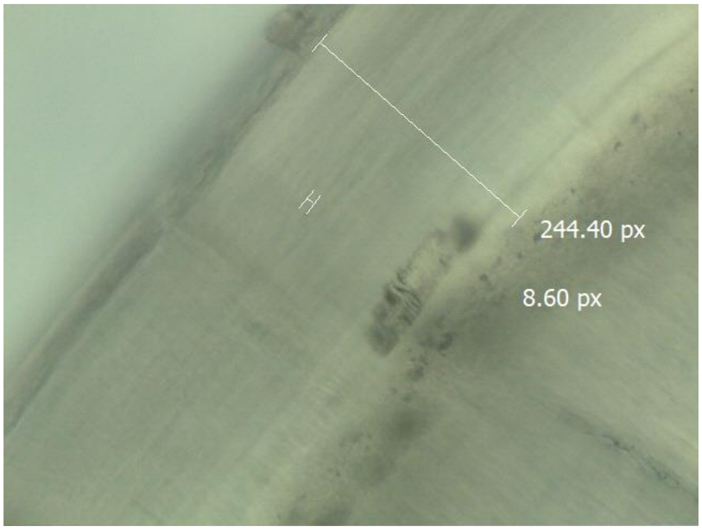

Figure 12: *Sample 22B, 1mL 87 Grade Gasoline 100x magnification with measurements*

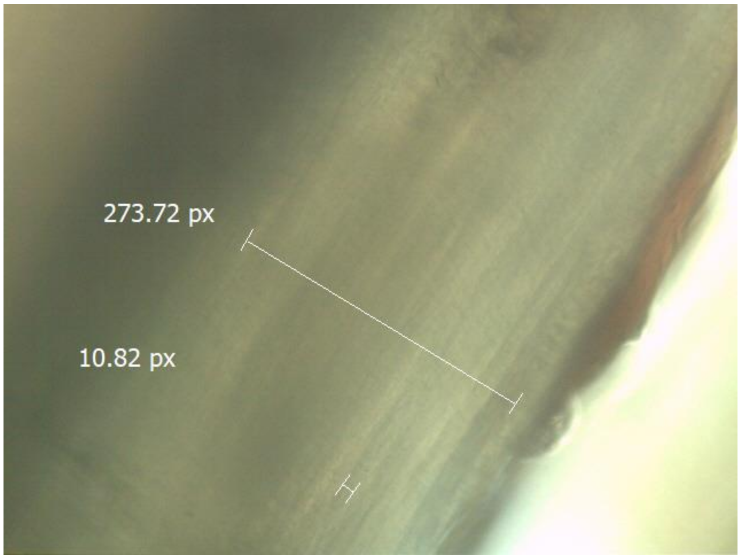

Figure 13: *Sample 11E1, 1mL Acetone 100x magnification with measurements*

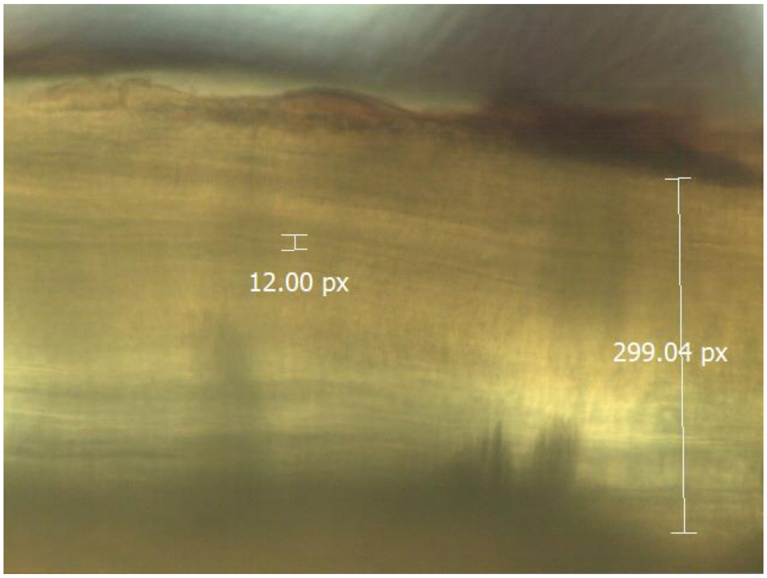

Figure 14: *Sample 18E3, 1mL Lighter Fuel 100x magnification with measurements*

The estimations from the equation were then documented via Microsoft® Excel® and were compared to the actual recorded ages of the individuals from whom the teeth were extracted. The range of tooth cementum annulations in relation to actual age ranged from 22.71 years to 86.89 years (see Figure 15).

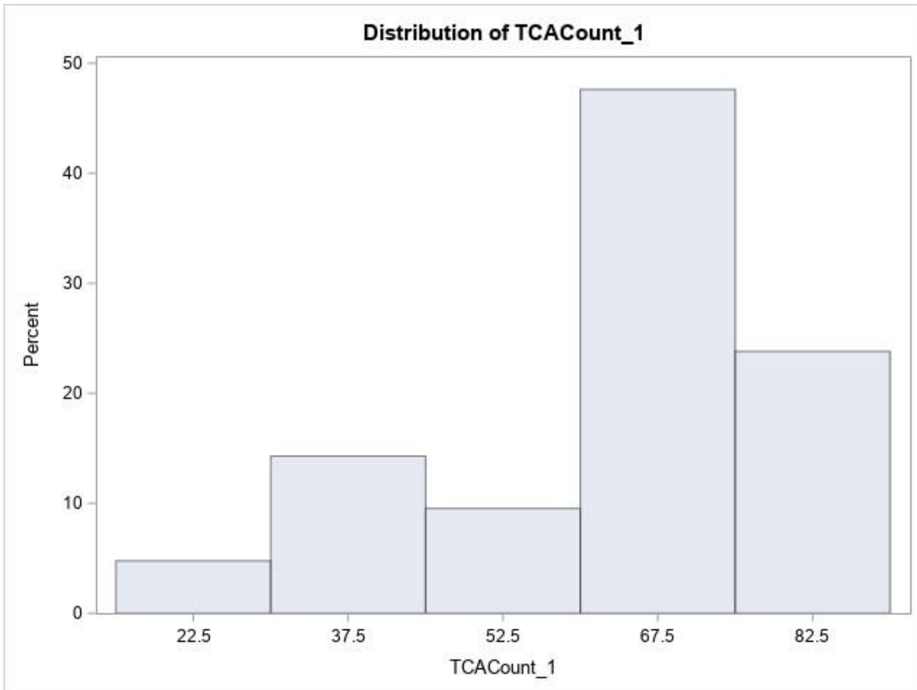

Figure 15: *Distribution of TCA count*

Data Analysis and Interpretation

The overall data was generated through photography via the Leica© LAS EZ Application Software. Data generated through the software was tracked using Microsoft® Excel®. Within the spreadsheet the sex of the individual, the type of tooth, the actual age of the individual, the type of accelerant, the final tooth cementum annulation count, the maximum temperature reached of the tooth, and the amount of time in seconds the tooth was subjected to alteration was documented.

This data was then transferred to SAS® Studio for analysis. Two tests were conducted to determine the significance of various variables within the study. An ANOVA test, or an analysis of variance test, is used to see if an experiment's results are significant in order to determine if there is a need to reject the null hypothesis or accept the alternate hypothesis. An ANOVA test was used to determine the statistics related to that of relevance of the sex of the individual and the type of tooth burned as well as the relevance of the type of accelerant used and the amount of accelerant used within the study. The second statistical test conducted for this experiment was a Pearson correlation coefficient test which is a measurement of the strength of a linear association between two variables. This test was used to determine if the novel approach to counting tooth cementum annulations was able to accurately determine the age of the individual from whom the tooth was extracted.

Results and Discussion

Following previous research, an ANOVA test was conducted to determine if there were any significant differences between male and female teeth and if that effected the ability to read tooth cementum annulations. The same statistical test was conducted to determine if there were any significant differences between the ability to read cementum annulations and the type of tooth that had been thermally altered. Knowing that there is noticeable sexual dimorphism within human dentition, these two variables were then also compared to each other to determine if there was any significant interaction between sex and tooth type. As seen in Table 3, the p-value of 0.0375 indicates that the model is significant and further tests were conducted to determine if the significance was due to the sex of the individual or the tooth type. As seen in Table 4, the Type III Sum of Squares (Type III SS, 2754.08) for tooth type indicates that the type of tooth extracted is the variable that impacts the ability to estimate the tooth cementum annulations and not the sex of the individual from whom the tooth was extracted.

Table 3: *ANOVA results with the P-Value 0.0375 to show significance between the sex of the individual versus the tooth type*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3097.475571	619.495114	3.18	0.0375
Error	15	2925.655153	195.043677		
Corrected Total	20	6023.130724			

Table 4: *ANOVA results showing that tooth type impacts the ability to read annulations*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sex	1	20.463045	20.463045	0.10	0.7505
Tooth_Type	3	2754.088358	918.029453	4.71	0.0165
Sex*Tooth_Type	1	97.058672	97.058672	0.50	0.4914

As seen in Figure 16, the data indicates that tooth cementum annulations are more clearly seen and read within incisors relative to that of molars. This may be due to the fact that teeth like incisors are single rooted where teeth such as molars are multi-rooted. During the cross-sectioning process, care was taken to section each tooth at the middle third of the tooth root. However, often times due to the quality of some of the teeth used throughout this study, the tooth roots were not fully intact, or the roots were in the progressive loss of dentine and cementum otherwise known as root resorption. This is turn could have potentially affected the ability to properly section the molars and in turn led to the inability to accurately see the tooth cementum. In summary, the sex of the individual had no impact on the ability to read tooth cementum annulations whereas tooth type did. However, no relationship was observed in conjunction with both the sex of the individual and the type of tooth that was thermally altered.

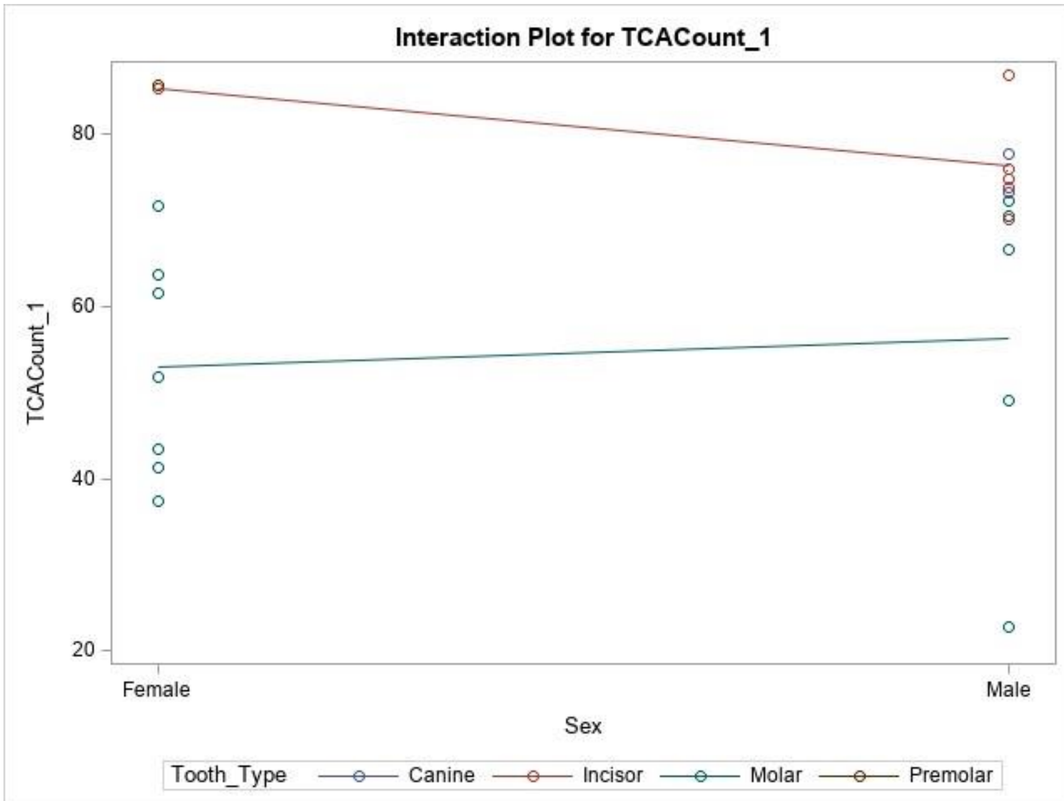

Figure 16: *Relationship between TCA count and tooth type*

Next, statistical analyses were conducted to determine if the accelerant type and if the accelerant amount impacts the ability to accurately read and count tooth cementum annulations. An ANOVA test found that the regression is significant (p-value 0.0298, see Table 5) and that there is a difference due to the accelerant type. As seen in Figure 17, the data indicates that if a tooth were exposed to acetone and thermally altered, the ability to count tooth cementum annulations were lower than those of lighter fuel and 87 grade gasoline. The wide range of tooth cementum annulation counts for acetone may be attributed to the age range of samples used for this accelerant. However, further research would need to be conducted with acetone which could provide more insight into this result.

Table 5: *P-Value 0.0298 showing that regression is significantly different*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1946.336690	973.168345	4.30	0.0298
Error	18	4076.794033	226.488557		
Corrected Total	20	6023.130724			

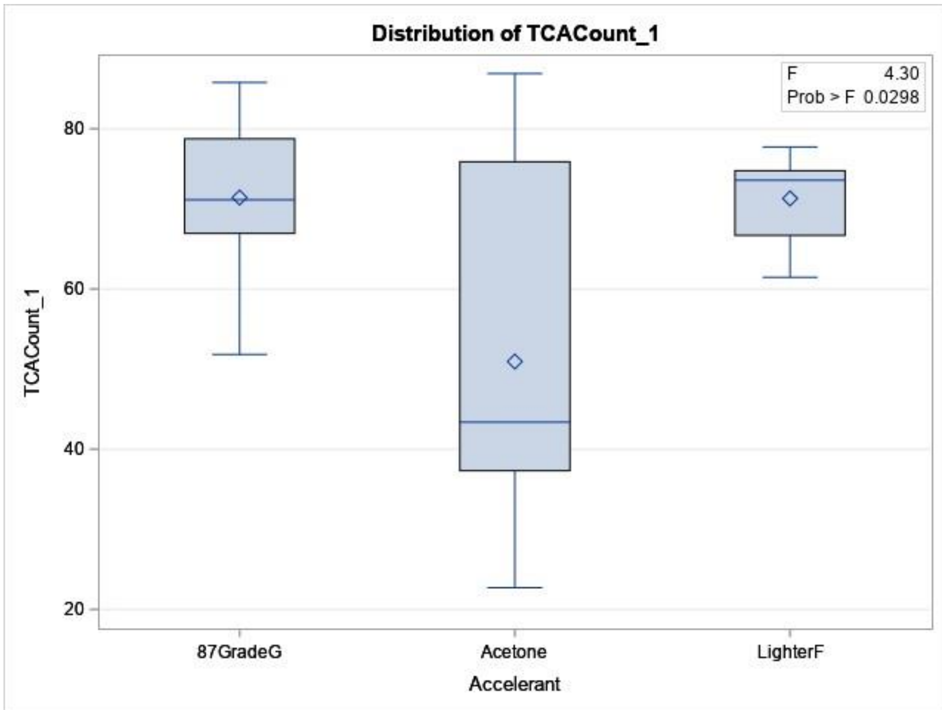

Figure 17: *Distribution of TCA counts to accelerant type*

The amount of accelerant used to thermally alter the samples was also statistically significant (see Tables 6 and 7). Table 7 shows that both the type of accelerant being used is significant with a 0.0017 p-value as well as the amount of accelerant being used with a 0.0201 p-value. However, with these statistics it was concluded that there was no relationship between the accelerant type and the amount of accelerant. Further investigation into this relationship is needed to better understand the phenomena and determine if this trend will remain.

Table 6: *P-Value 0.0119 showing the model is significant in the relationship between the type of accelerant and the amount of accelerant*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	4950.248524	495.024852	4.61	0.0119
Error	10	1072.882200	107.288220		
Corrected Total	20	6023.130724			

Table 7: *Type III SS and P-Value showing that both type of accelerant and accelerant amount impact the ability to read cementum annulations*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Accelerant	2	2751.683137	1375.841568	12.82	0.0017
Accelerant_Amount	3	1676.689097	558.896366	5.21	0.0201
Acceleran*Accelerant	5	1262.984186	252.596837	2.35	0.1168

Figure 18 demonstrates that with smaller amounts of accelerants, 1mL specifically, annulations are more easily read and visualized. However, annulations were still able to be read in a few samples with 4mL. Teeth that were thermally altered in acetone yielded the lowest number of annulations that were able to be read. However, both 87-grade gasoline and lighter fuel yielded similar counts with the same volume.

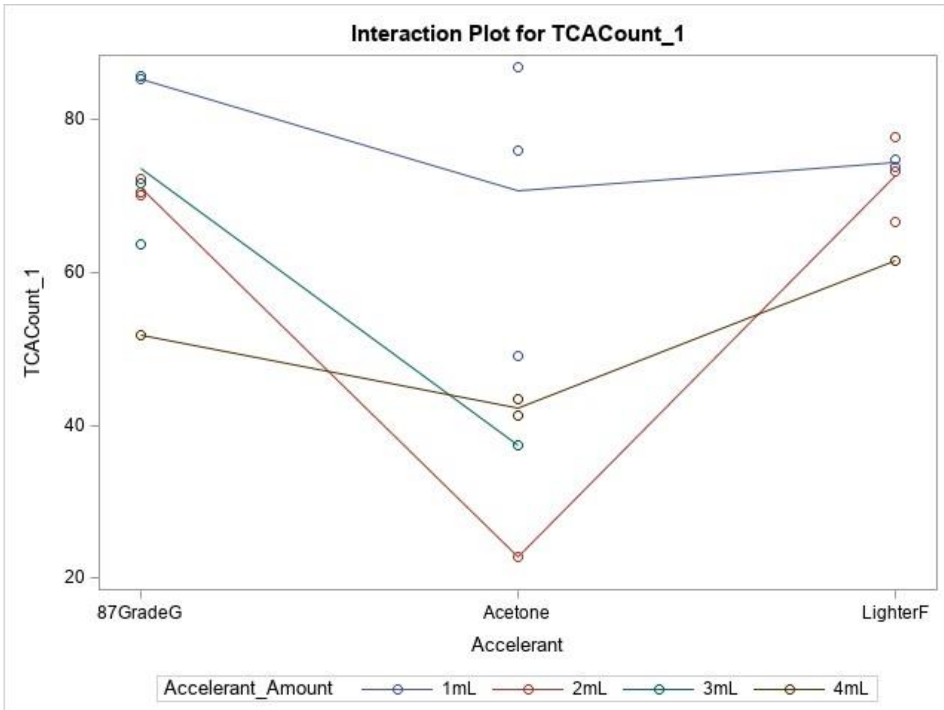

Figure 18: *Interaction plot between type of accelerant and TCA count*

Lastly, a Pearson Correlation Coefficient test was conducted to look at the relationship between the actual age of the individuals and the age estimations that were completed with the two-step formula created within this study. Table 8 shows that the correlation between actual age of the individual and tooth cementum annulation count is significant at 0.94485.

Table 8: *Pearson Correlation Coefficient P-value 0.94485 showing that there is a strong correlation between estimated age and actual age*

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations	
	Actual_Age
TCACount_1	0.94485 <.0001 21

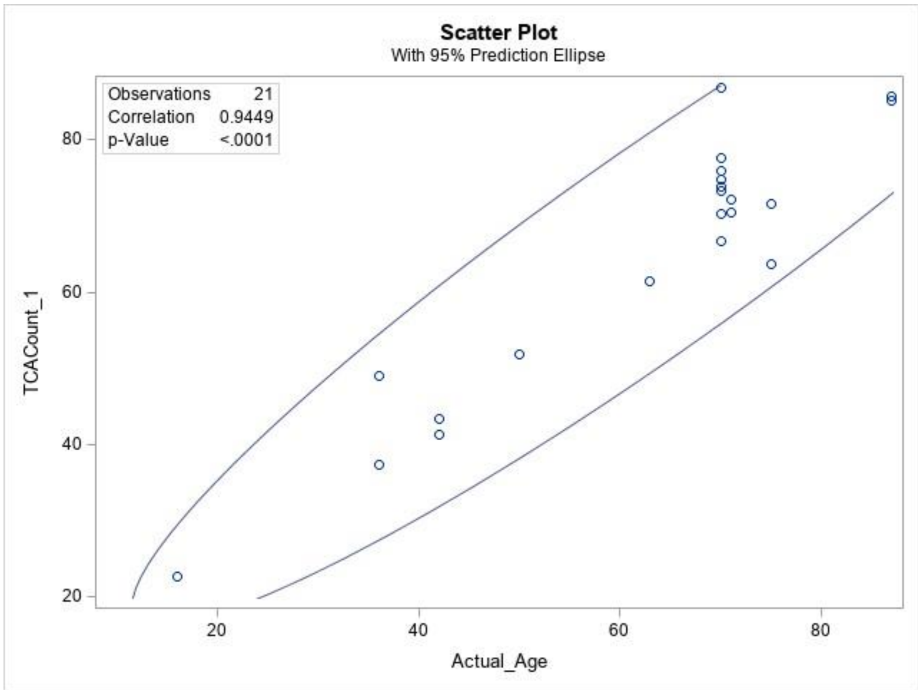

Figure 19: *Scatter plot showing correlation between actual age and TCA count*

The scatter plot above, Figure 19, indicates that this novel estimation method was successful. The new two-step counting method was able to accurately estimate age even with numerous variables contributing to alteration. Despite the accelerant alteration, accurate age estimations were completed and are highly correlated with the actual age of the individual from whom the teeth were extracted from. These results are not impacted by the biological sex of the individual; however, the type of tooth does impact the ability to read the cementum areas but did not inhibit the ability to correctly assess age entirely based on tooth structure.

Conclusion

Due to the data results, it can be concluded that tooth cementum annulations can still be an aid in age estimations when thermal alteration via accelerants is present. With there being no difference between the sexes, this method can apply to all individuals within these situations.

This method, if applied correctly and if using the correct equipment, could save forensic anthropologists an immense amount of time when estimating age in an unknown individual. This method could shrink the age range that is often broad and insufficient compared to the smaller age ranges that cementochronology produces. This is exceedingly helpful in scenarios where multiple deceased individuals of similar age ranges are present. By being able to apply the more precise method, the ambiguity of traditional age determinations can be eradicated.

For future research, there are a few modifications that can be made to allow for this study to be more attainable, especially if the resources outlined by Colard et al are not readily available. First, the saw in this study was not able to thin section each sample that was encased in resin. Rather these samples were sectioned to approximately 1-2cm thick and then were hand thinned with sandpaper. In order to save time, obtaining a saw that is able to thin section each sample would be preferred. If not available, sending the samples to a professional company to be thinned would be ideal. While this is not the most financially probable option, it will save on time and preserve the integrity of the samples more so than hand thinning with sandpaper. Lastly, obtaining a more powerful microscope would allow for visualization of the darker samples, such as the ones that were burnt in 4mL of an accelerant. In Gocha and Schutkowski's 2013 study, they were able to observe annulations that had been altered up to 600 degrees Celsius. Comparatively, the highest temperature achieved in this study was 229.6 degrees Celsius and many of those teeth were unable to be visualized due to the severity of burning. This inability is likely due to the power of the microscope used, and not being able to get the samples thin enough to see through, or due to the fact that direct flame is more destructive than that of indirect heat. Future studies looking at each of these limitations would provide more insight to the use of this method.

This study and future studies to come dedicated to tooth cementum annulations will have a great impact of the field of forensic science and forensic anthropology in regard to age-at-death estimations. In terms of this study, it is now known that cementochronology can be used in mass disaster situations, accidents, and homicides where accelerants play a role in the destruction of the remains of the victim and/or victims. This study was limited to only one observer estimating age through the new technique which can potentially lead to inconsistencies or inaccuracies in the estimation method. For future studies, the number of observers should be expanded, and the estimations taken from each observer should be compared to see if the age estimation method continues to be accurate. Estimations of the same samples between observers should also be researched to ensure reproducibility. As well, this study demonstrated limited supplies and instrumentation compared to Colard et al. can still be implemented and applied to smaller laboratories with limited resources. Cementochronology and the use of tooth cementum annulations for age estimations should be a method used more often within forensic anthropology research and identification laboratories.

For future projects dedicated towards the use of cementochronology in forensic contexts, different alteration methods should be considered and explored further. As noted in previous research, the ability to read cementum annulations in thermally altered teeth is possible but looking at further accelerants beyond those used in this research has not been explored. Future accelerants to consider could include aviation fuel, varying grades of gasoline, or other household flammable liquids like ethyl ether or paint thinner. Due to the limitation of time, lack of specimens, and shortage and unavailability of supplies this research was restricted to only utilizing three accelerants. As well, alteration methods such as the use of acids and bases are often involved in the removal of evidence and occasionally the disposal of remains. Looking to see if annulations can still be read after being subjected to long periods of time in varying chemicals such as hydrochloric acid, bleach, and lye could be of use in forensically significant cases.

In regard to sample size, having access to a larger number of teeth could show varying results as well. In this research, it was found that the type of tooth did attribute to the ability to read tooth cementum annulations. Dedicating a study to observing trends based on which teeth to specifically utilize in thermal altering situations may also be of forensic importance when performing real casework. With the implementation of a new method of counting tooth cementum annulations, this method needs to be tested to ensure its accuracy. Future studies should be dedicated to practicing with this method both on unaltered teeth and on teeth that have been altered in various ways. Lastly, potentially having access to a larger area to perform the thermal alterations can more accurately mimic real-life scenarios. This research was conducted in an indoor laboratory and very small amounts of accelerants were used. If this could be safely conducted outdoors, larger volumes of accelerants could be used to more accurately mimic thermally altering scenarios.

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